

The present application claims priority to US Provisional Patent application serial Number 60/097,210, filed August 20, 1998 and US Provisional Patent application serial number 60/141,169, filed June 25, 1999.

INS 12  
Field of the Invention

The present invention relates generally to the field of cancer treatments, as well as to the field of peptide and non-peptide pharmaceutical compounds.

1003566-122801  
BACKGROUND OF THE INVENTION

Many lung and prostate cancers, of which small cell lung cancer (SCLC) is a prime example, have a neuroendocrine phenotype, and their growth is stimulated by neuropeptides. Antagonists of several peptides (e.g. bradykinin, substance P, bombesin) have been used in experimental treatment of models of SCLC in animals. Among the most potent of the peptides examined thus far, crosslinked dimers of certain bradykinin antagonist peptides have been efficacious both *in vitro* and *in vivo* against strains of SCLC and other tumors (Chan et al., *Immunopharmacology* 33: 201-204, 1996; Stewart et al., *Can. J. Physiol. Pharmacol.* 75: 719-724, 1997; Stewart et al., US Patent Application 5,849,863, issued 12/15/98). Prostate cancers show a similar neuroendocrine phenotype and are susceptible to neuropeptide antagonists.

SUMMARY OF THE INVENTION

30 The present invention provides anti-cancer agents (ACA) comprised of a range of novel amino acid derivatives and small peptides having the ability to inhibit growth of SCLC and certain other tumor cell lines (such as non-small cell lung cancer (NSCLC) and prostate cancer) in standard *in vitro* tests, as well as certain monomeric peptides that show inhibition of tumor growth *in vivo*. Certain of the peptides have a general structural relationship to carboxy-terminal

5 fragments of bradykinin antagonists, but the non-peptides show no such general relationship. Monomers, dimers, trimers, tetramers, pentamers and cyclized analogs of the novel molecules are described. The new compounds are tested for bradykinin antagonist activity in standard assays, but there is no apparent relationship between bradykinin antagonist activity and cytolytic potency. All of the molecules described possess both hydrophobic (usually aromatic) and basic  
10 groups in their structures. Without being held to one particular theory, it appears that the compounds function by stimulation of cell death (apoptosis) in the tumor cells.

The present invention also provides compounds and methods for inhibiting cancer by administering to a subject afflicted with cancer (ie. of the lung or prostate) a therapeutically effective amount of one or more of the compounds herein described.

In general, the anti-cancer compounds are described by the formula:



wherein X is a linker having 2-5 functional groups or is absent, n = 1-5, and ACA is selected from the group consisting of Formula II, Formula III, Formula IV, Formula V, and Formula VI. Other compounds described herein are defined by the Formula VII. The specifics regarding structure are enumerated in the Detailed Description, Examples and Claims. Certain physical characteristics are enumerated in the Examples as well as the Detailed Description, Examples and Claims.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by B10054.

25 Figure 2 shows inhibition of growth *in vivo* of NSCLC strain A-549 by M620.

Figure 3 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by B9430.

Figure 4 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by B10238

Figure 5 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by M570, both as the trifluoroacetate salt and as the hydrochloride salt.

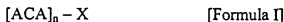
30 Figure 6 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by M822.

Figure 7 shows inhibition of growth *in vivo* of SCLC strain SHP-77 by M638.

# DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a range of monomeric, dimeric, trimeric, tetrameric, pentameric and cyclic small peptides and peptide mimics that are effective as anti-cancer agents.

In general, the anti-cancer agents (ACA) are described by the formula:



wherein X is a linker group having 2-5 functional groups or is absent,  $n = 1$ , and ACA is selected from the group consisting of Formula II, Formula III, Formula IV, Formula V, and Formula VI, as described herein. Other compounds described herein are defined by the Formula VII, as described herein.

X can be any linking group which does not interfere with the inhibitory activity of the monomer-linker or oligomerized product using *bis*-imido-esters, *bis*-maleimidoalkanes such as *bis*-maleimidohexane, dicarboxylic acids, tricarboxylic acids, tetracarboxylic acids and multi carboxylic acids. Alkane groups may be substituted with alkyl, amino, carboxyl, halogen, hydroxy, mercapto or methoxy groups. Aminoalkyl, aromatic or cycloalkyl polycarboxylic acids, heterocyclic polycarboxylic acids, carboxylic anhydrides and polyoxyethylene linkers may also be used. For C-terminal crosslinking, X may be any diamino or polyamino alkane, cycloalkane, aromatic, heterocyclic diamine, polyamine or other substituted chelating agent (for example: diethylenetriaminepentaacetic dianhydride, ethylenediaminetetraacetic dianhydride, etc.). Polyamino-polycarboxylic acids may also be used to make heteromers (such as ethylenediamine-N,N'-diacetic acid, etc.).

The linkage may be at the N-terminal or the C-terminal or at any position of the ACA sequence through side-chain functional groups. The linker may have any chain length.

For dimers, there is a correspondence between linker length and cytotoxicity. Alkyl chains of 8 carbons or more are preferred, with those of 8 to 18 carbons being most preferred. Examples of preferred dimer linkers for the  $\alpha$ -amino at the N-terminal or for a basic side-chain

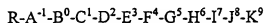
5 group at any position of ACA include ADA, BTAC, DDD, DDS, DTP, EGS, EOPC, HDD, HFG, PFS, SBEC, SUB, SUIM and TDIM. For dimerization through the C-terminal carboxyl or any side-chain carboxyl in ACA, the preferred linkers include DDA, DEA, EDA, EDP and HAD. Any di-functional molecule can be used.

10 For trimers, linkers for basic groups include BTAC, BTC, CHTC, CTAC and TREN-( $\text{Suc}$ )<sub>3</sub>; for carboxyl groups, TREN. Any tri-functional molecule can be used.

For tetramers, linkers can be BAPTA, CPTA, EDTA, EGTA, ETТА, or any tetra-functional molecule.

For pentamers, the linker can be DTPA or any pentameric functional molecule. Compounds formed by ACA and a linker X may be homo or hetero multimers.

[Formula II] comprises:



wherein R, A, B, C, D, E, F, G, H, I, J, and K are selected from the following or may be absent, and wherein K is Arg or an Arg derivative:

R	A	B	C	D	E	F	G	H	I	J	K
	-1	0	1	2	3	4	5	6	7	8	9
Absent or 3,3DP Aaa Ac	Absent or DmK Lys Lys(eF lu)	Absent or Apc Arg DArg	Absent or ApC Arg DmK	Absent Or MeP Nig NMF	Absent or Hyp Pro	Absent or Ava BAla Dpr	Absent or Add Aud CpG	Absent or Arg Gly Pac	Absent or 2Nal DCpG DF5F	Absent or 2Nal 2Nal- NH <sub>2</sub>	Absent or Arg Arg(H) Arg- CH <sub>2</sub> O H
Aca	NiK	DLys	NiK	Pro		Eac	DDMF	Pac	DIgl	3,4F2F	Arg- NH <sub>2</sub>
BApp	PzO	DmK	NiO			Gly	DMF	Ser	DPFF	3Pal	Arg(N O <sub>2</sub> )
Cca		DniK	PaF				Eac	Thr	DPhe	Ac6c	Arg- OMe
Cin Dca		DpaF DPzK	PzO				Igl Lys		DTic Gly	Aic Ana	DArg DArg- NH <sub>2</sub>
Deg		DPzO					Pac		mABz	Apb	DArg( NO <sub>2</sub> )
Dhq		Lys					Phe		pABz	Apb	

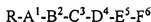
10035667.122801

Dmac Dpa	NiK PaF	Thi	Pac PaF(Dc g) pAmb	Atpe Bip Cmp CpG
F5bz	PzO			DhPhe
F5c	DArg- (NO <sub>2</sub> )	Arg- (NO <sub>2</sub> )		Dpr(Fb z) Dpr(Pa a) F5F F5F- NH <sub>2</sub> Hphe Ica Igl Igl- NH <sub>2</sub> Ileu Lys(C H <sub>3</sub> ) <sub>3</sub> Lys(F5 bz) Mapa MBC MFF Nc6G Nc7G NMF OBS OBT OBY OC2Y Oic Oic- NH <sub>2</sub> PABz Pac PaF(F5 c) PaF(Fb z) PaF(M cg) PaF(Pp a) PaF(Si n) pAmb pAPa PCF
F5pa				
Gun				
Hxa				
Mca				
Mcg				
Moti				
Pcc				
Ppa				
Pya				
Saa				
Ste				
Tfinc				

PdF  
PFF  
PFF-  
NH<sub>2</sub>  
Phe  
PNF  
Thi  
Tic  
Trp  
Trx  
Tyr

5.

[Formula III] comprises:



wherein R, A, B, C, D, E, and F are selected from the following or may be absent, and wherein F is not Arg or an Arg derivative:

R	A 1	B 2	C 3	D 4	E 5	F 6
Absent or 2,2Dp 3,3Dp	Absent or DArg DArg(NO <sub>2</sub> )	Absent or Arg	Absent or Add Aud	Absent or 2Nal 3Pal	Absent or 1Nal 2Nal	Absent or 2Nal 3Pal
Aaa			Ava	Arg	2Nap	ABza
Ac			Eac	Arg(Tos)	3Pal	ABza
Aca			Lys	Atcp	Apa	Ama
Boc			Pac	D2Nal	Arg	Ampy
Chc				DArg	Arg-NH <sub>2</sub>	Ampz
Cin				DArg(Tos )	Asp	Apa
Ctim				DF5F	Atc	Api
Dca				Dlgl	Atcp	Aptp
Dcg				DPFF	Bip	Aqd
Dhq				Eac	BtA	Aqu
Dmac				F5F	Cys(Meb)	Arg(H)
Dns				Gly	Cys(SO <sub>3</sub> H )	Arg- CH <sub>2</sub> OH
Dpa				His	D2Nal	Arg-NH <sub>2</sub>
F5c				Igl	DArg	Arg-OMe
F5pa				mABz	DArg-NH <sub>2</sub>	Asp
F5po				OC2Y	F5F	Asp(Aqu)
Gbc				Pac	Glu	Atcp
Gun				PFF	Gly	Atmp
Hxa					Igl	AtmpO
Mcg					Inp	Atpm
Mse					Iqa	Cyh

0035662.122801

10035662-122801

Py  
Se  
Sin

Sul  
Tfmc  
Tha

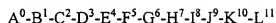
mABz  
MC2Y  
N-Dmb-  
Tyr(Bz)-  
OMe  
OC2Y  
OCIY  
Oic  
pABz  
PaF(Mes)  
PFF  
Tic  
tLeu  
Trp  
Try  
Try(Bzl)  
Tyr  
Arg(NO<sub>2</sub>)  
pABz  
PaF  
PaF(Dcg)  
PaF(Mcg)  
PaF-NH<sub>2</sub>  
PFF-NH<sub>2</sub>  
PgF  
PzO  
Sud  
Thm  
Thm  
Tpac  
Tpac  
Tyr(Bz)O  
Me

Dmab  
Dmm  
Dmp

Dpea  
Dpma  
Dpr(Dcg-2-  
Nap)  
Ecap  
F5F-NH<sub>2</sub>  
GaP  
m<sub>A</sub><sub>2</sub>Bz  
m<sub>A</sub><sub>2</sub>Bz(Dc  
g)  
m<sub>A</sub><sub>2</sub>Bz(Gu  
n)  
mABz  
Mapp  
Matp  
MatpO  
pABz  
PaF  
PaF(Dcg)  
PaF(Mcg)  
PaF-NH<sub>2</sub>  
PFF-NH<sub>2</sub>  
PgF  
PzO  
Sud  
Thm  
Thm  
Tpac  
Tpac  
Tyr(Bz)O  
Me

5

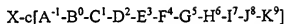
[Formula IV] comprises:



10 wherein A, B, C, D, E, F, G, H, I, J, K and L are selected from the following or may be absent:

A	B	C	D	E	F	G	H	I	J	K	L
0	1	2	3	4	5	6	7	8	9	10	11
Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
or	or	or	or	or	or	or	or	or	or	or	or
DArg	Arg	Pro	Lys	Pro	DTrp	Gln	DTrp	Phe	DTrp	Leu(r)	Leu- NH <sub>2</sub> Leu
DArg						DNMF					

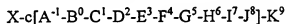
[Formula V] comprises:



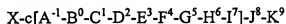
10 wherein X, A, B, C, D, E, F, G, H, I, J, and K are selected from the following or may be absent:

X	A	B	C	D	E	F	G	H	I	J	K
	-1	0	1	2	3	4	5	6	7	8	9
Absent or $\alpha$ -Aca 3,3Dp	Ava BAIa DmK Glt Lys Suc	DArg DNik DPaF DPzK DPzO	Arg NiK PzO	Pro	Hyp	Gly	Add Aud Ava BAIa DNMF Eac Igl Thi	DArg Ser Thr	DDab DDpr DF5F DIgl DLys DOm DPaF Nig Pac Phe	DTTrp F5F Lys Nc7G Oic PaF PFF Phe	Arg Leu NiK PaF 3Pal

[Formula V] also comprises:

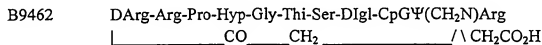
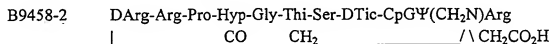


[Formula V] also comprises:



20 wherein the cyclization is via a side chain functional group other than the C-terminal residue and the residues are as described in the immediately preceding table.

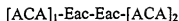
[Formula VI] comprises the following cyclic peptides:



ACA can also be those compounds in Table 4.



[Formula VII] comprises:



wherein [ACA] is defined by Formula I or the compounds in Table 4.

The *in vivo* inhibitory effects of antagonists may be studied using tumor-bearing nude mice. A tumor model employing nude mice orthotopically implanted with human lung cancer cells wherein the ACA is delivered by intratracheal instillation and aerosol inhalation may be used to evaluate the efficacy and feasibility of these antagonists as a means of treating human lung cancers. Control animals without tumor implantation may also be used to study the general side effects or cytotoxicity of the compounds. It is believed that aerosolized delivery or intratracheal instillation of the agents can produce effective dose accumulation in the area of lesion and reduce the overall systemic toxicity of the compounds in the animals more than when the compound is delivered by systemic administration.

The compounds may be administered topically, or by injection or infusion or as an oral suspension in an appropriate vehicle or as tablets, pills, capsules, caplets or the like, or preferably via intratracheal instillation or aerosol inhalation. The dosage and manner of administration will be defined by the application of the ACA and can be determined by routine methods of clinical testing to find the optimum dose. These doses are expected to be in the range of 0.001 mg/Kg to 100mg/Kg of active compound.

The compounds are composed of amino acids which may form salts due to their acidic or basic nature, and any pharmacologically acceptable salt derived from the compounds described in this invention such as hydrochlorides, acetates, phosphates, maleates, citrates, benzoates, salicylates, succinates, ascorbates and the like, including HCl, trifluoroacetic acid (TFA), and HOAc, are considered an extension of this invention. A common tactic in medicinal chemistry is to modify known drug substances which are peptide based to form esters or amides which exhibit greater bioavailability. Prodrugs derived from the compounds disclosed here are

10035662-122801

5 therefore considered an obvious extension of this invention. Methods for designing and preparing prodrugs are described in detail in the medicinal chemical literature.

Structures and biological activities of peptides and peptide mimics related to bradykinin (BKR) are given in Table 1. Structures and biological activities of compounds not related to bradykinin (BKU) are given in Table 2. Structures and biological activities of cyclic peptides are given in Table 3. Structures of previously described known peptides which we have found to be active against cancers *in vivo* are included in Table 4. Actions of selected compounds on prostate cancer cell lines are given in Table 5. Abbreviations used are as defined in Table 6.

## EXAMPLES

In general, Anti-bradykinin activity was determined by the classical guinea pig ileum assay and on Chinese hamster ovary (CHO) cells expressing cloned human bradykinin B2 receptors. Anti-tumor activity was determined on cultured human cancer cell lines using the standard tetrazolium (MTT) assay. No correlation between anti-bradykinin and cytolytic activity was found among the compounds, indicating that cells are not killed due to inhibition of an essential bradykinin function. Potent compounds were found to stimulate apoptosis in SCLC cells, probably by abnormal activation of the intracellular MEKK pathway.

### EXAMPLE I – Synthesis of peptides

Peptides were synthesized using standard solid phase synthesis methods, well known in the art (Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL, 1984) and were purified by HPLC and were characterized by amino acid analysis (AAA), thin layer chromatography (TLC) and laser desorption mass spectrometry (LDMS). Peptide amides were synthesized on methylbenzhydrylamine (MBHA) resin, which yields amides directly. Peptide methyl esters (OMe) were synthesized by reaction of peptides with 2,2-dimethoxypropane (Rachele, *J. Org. Chem.* 28: 2898, 1963). Cyclic peptides were prepared on resin or in solution with PyAOP and HOAt .

**EXAMPLE II – Synthesis of non-peptides**

Non-peptides were synthesized by standard organic chemistry procedures well known in the art. Compounds were purified by HPLC and were characterized by analytical HPLC, TLC, and LDMS.

**EXAMPLE III – Synthesis of DDD and SUB dimers**

Synthesis on resin: Neutralized peptide-resin (0.05mmole) was treated with 0.15 mmole diisopropylethyl amine (DIEA) and 0.026 mmole dodecanedioyl dichloride or suberoyl dichloride in 2.5 mL dichloromethane (DCM). The suspension was mixed for 5 h, washed with DCM and ethanol and dried. The peptide dimer was cleaved from the resin with HF, and the peptide was extracted and purified

Synthesis in solution: Carboxyl-derivatized amino acids or dipeptides were dissolved in dimethyl formamide (DMF) and treated with 10 equivalents of DIEA and 0.55 equivalent of dodecanedioyl dichloride or suberoyl dichloride overnight. The DMF was evaporated *in vacuo* and the resulting dimer was purified by HPLC.

**EXAMPLE IV – Synthesis of EGS, DTP, SBEC and SUB dimers in solution**

Dimerization in solution proceeded by reacting 1 equivalent of peptide monomer trifluoroacetate, an excess of DIEA and 0.55 equivalent of cross-linking reagent overnight in DMF. The cross-linking agents were purchased from Pierce (EGS dimer, ethylene glycol *bis*-(succinimidylsuccinate); DTP dimer, dithio*bis* (succinimidyl propionate); SBEC dimer, *bis*[(2(succinimidooxycarbonyloxy)ethyl)sulfone]; SUB dimer, disuccinimidyl suberate).

5 **EXAMPLE V – Synthesis of Boc-N-cycloheptylglycine (Nc7G)**

N-Cycloheptylglycine was synthesized by reductive amination of cycloheptanone with glycine methyl ester following the procedure described in Gera *et al.*, *Immunopharmacology*. 33:174-177 (1996). The crude product was converted to the N-Boc derivative (mp, 89-90 °C).

10 **EXAMPLE VI – Synthesis of TDIM dimers**

Dimethyl tetradecyldiimide was synthesized from tetradecanedinitrile by the method of De Abreu *et al.* (*Eur. J. Biochem.* 97: 379- 387, 1979). One equivalent of peptide TFA salt or other molecule having a free amino group was dissolved in DMF and stirred with 10 equivalents of DIEA and 0.7 equivalent of dimethyl tetradecyldiimide dihydrochloride overnight at room temperature. DMF was evaporated *in vacuo* and the dimer was purified. SUIM dimers were prepared similarly, using dimethyl suberimide.

**EXAMPLE VII – Synthesis of B10238: F5C-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg (F5c-B9430)**

B10238 was made by standard solid phase synthesis procedures, or by the acylation of B9430 with 2,3,4,5,6-pentafluorocinnamic acid in DMF, using BOP coupling in presence of excess DIEA. The product was purified by HPLC.

**EXAMPLE VIII – Synthesis of M822: DDD-(DArg-F5F-Arg)<sub>2</sub>**

Following standard solid phase synthesis procedures, Boc-Arg(Tos) Merrifield synthesis resin was coupled with Boc-F5F, followed by coupling with Boc-DArg(Tos), using HATU as coupling agent. The peptide-resin was deprotected with TFA-DCM and neutralized with TEA. The peptide-resin was then treated with 0.55 equivalent of dodecanedioyl dichloride and 5 equivalents of DIEA in DCM overnight at room temperature. After washing and drying, the resin was cleaved with anhydrous HF, using standard conditions. The peptide was extracted from the resin with 90% acetic acid and purified by preparative HPLC.

**EXAMPLE IX – Synthesis of M570 Hydrochloride: F5c-OC2Y-Atmp.HCl**

4-Amino-2,2,6,6-tetramethylpiperidine (Aldrich) was coupled with Boc-(O-2,6-dichlorobenzyl)-tyrosine, using BOP in DMF solution. The Boc protecting group was removed by TFA and the product coupled with 2,3,4,5,6-pentafluorocinnamic acid in DMF, using BOP in the presence of excess DIEA at room temperature for 3 h. The DMF was removed *in vacuo*, the product was extracted into ethyl acetate and the solvent was evaporated. The residue was treated with 0.1 - 1.0 N HCl or 20% ethanolic HCl. The solvent was removed by evaporation *in vacuo* at room temperature. The residue was lyophilized from water-dioxane or crystallized from ethanol-ether.

**EXAMPLE X – Synthesis of M630: Dmac-OC2Y-Matp.TFA**

4-Methylamino-2,2,6,6-tetramethylpiperidine (Matp) was synthesized from 2,2,6,6-tetramethyl-4-piperidone (Aldrich) and methylamine by reductive amination with NaCNBH<sub>3</sub>. The Matp was coupled with Boc-(O-2,6-dichlorobenzyl)-tyrosine, using BOP in DMF solution. The Boc protecting group was removed by TFA and the product was coupled with 4-(dimethylamino)cinnamic acid in DMF, using BOP in the presence of excess DIEA at room temperature for 3 h. The DMF was removed *in vacuo*. The product was extracted into ethyl acetate and the solvent was evaporated *in vacuo*. The crude product was purified by HPLC, giving the TFA salt. The Dmac-OCTY-Matp.TFA salt can be converted to its HCL salt as in Example IX above.

**EXAMPLE XI – Synthesis of M638: DDD-(DArg-Igl-Arg-Matp)<sub>2</sub>**

In sequence, Boc-Arg(Tos), Boc-Igl and Boc-DArg(Tos) were coupled to 4-methylamino-2,2,6,6-tetramethylpiperidine (Matp), using BOP as coupling agent in DMF in the presence of excess DIEA at room temperature for 3-5 h. After removal of DMF *in vacuo*, the product was extracted into ethyl acetate. After evaporation of the solvent, the residue was treated with TFA-

5 DCM to remove the Boc group. TFA was removed *in vacuo*. The DArg(Tos)-Igl-Arg(Tos)-  
Matp.TFA was treated with dodecanedioyl dichloride (0.55 equiv) and DIEA (5 equiv) in DCM  
for 5 h. The protecting groups were cleaved by HF and the lyophilized product was purified by  
HPLC. The M638.TFA salt was converted to its HCl salt, using 0.1 - 1.0 N HCl or 20%  
ethanolic HCl as in Example IX above.

10

#### EXAMPLE XII – Synthesis of M590: Atmp-Igl-Pac- $\alpha$ -Sbl-Lys-B9430

In sequence, Boc-Igl, Boc-Pac and mono-methyl sebacate were coupled to 4-amino-  
2,2,6,6-tetramethylpiperidine (Atmp), using BOP coupling agent in DMF in presence of excess  
DIEA at room temperature for 3-5 h. DMF was removed *in vacuo* and the product was extracted  
into ethyl acetate. After evaporation of the solvent, the methyl ester was hydrolyzed in methanol  
by 1N NaOH. The crude product (0.025 mmol Atmp-Igl-Pac-Sbl) was coupled to the peptide  
resin (0.02 mmol Lys(2-ClZ)-DArg(Tos)-Arg(Tos)-Pro-Hyp-Gly-Igl-Ser(Bzl)-DIgl-Oic-  
Arg(Tos)-Merrifield resin) using BOP/DIEA activation in DMF. The heterodimer peptide was  
cleaved from the resin with HF, using standard conditions. The peptide was extracted from the  
resin with acetic acid and purified by preparative HPLC.

1035662-10301

#### EXAMPLE XIII – Synthesis of M872: c[DArg-Arg-Eac-Ser-DF5F-Oic-Arg]

Following standard solid phase synthesis procedures, Boc-DArg(Tos) was coupled to  
Boc-Arg(Tos) Merrifield synthesis resin, followed in sequence by Boc-Arg(Tos), Boc-Oic, Boc-  
25 DF5F, Boc-Ser(Bzl), and Boc-Eac, using HATU as coupling agent. After deprotection with  
TFA-DCM, the resin was cleaved with anhydrous HF using standard conditions. The peptide  
was extracted from the resin with 0.1% TFA-H<sub>2</sub>O/dioxane and lyophilized. The peptide  
trifluoroacetate was cyclized with three equivalents of PyAOP and HOAt and 20 equivalents of  
DIEA in DMF at a concentration of 10<sup>-3</sup> M. After removal of the solvent under reduced  
30 pressure, the product was lyophilized from dioxane-H<sub>2</sub>O and purified by HPLC.

5 **EXAMPLE XIV – Synthesis of M678: (Dns-DArg-Igl-Arg)<sub>2</sub>-DDA**

In sequence, Boc-Arg(Tos), Boc-Igl and Boc-DArg(Tos) (2 equivalents) were coupled to 1,10-decanediamine using BOP as a coupling agent in DMF in presence of excess DIEA at room temperature for 3-5 h. DMF was removed *in vacuo* and the product was extracted into ethyl acetate. The solvent was evaporated *in vacuo* and the residue was treated with TFA/DCM to  
10 remove the Boc group. TFA was removed *in vacuo*, and the product was treated with dansyl chloride (2 equivalents) and an excess of DIEA in DCM for 5 h. The Tos groups were cleaved by HF and the crude product was purified by HPLC.

**EXAMPLE XV – Synthesis of M290: BTAC-(2-Nal-Atmp)<sub>3</sub>**

The benzene-1,3,5-*tris*-carbamido-ε-caproic acid linker was made from 1,3,5-benzenetricarboxylic acid and N-Boc-ε-caproic acid methyl ester, using the BOP coupling method. The methyl ester was hydrolyzed in methanol by 1N NaOH. The product (1 equivalent BTAC) was coupled to 2-Nal-Atmp (3 equivalents) in DMF, using HATU as coupling agent. The solvent was removed *in vacuo*, and the residue was purified by HPLC. The BTAC-(2-Nal-Atmp)<sub>2</sub>-OH was also isolated as a by-product.

**EXAMPLE XVI – Synthesis of M1040: EDTA-(OC2Y-ATMP)<sub>4</sub>**

Boc-(O-2,6-dichlorobenzyl)-tyrosine was coupled with 4-amino-2,2,6,6-tetramethylpiperidine overnight in DMF, using BOP as coupling agent in the presence of DIEA.  
25 After removal of DMF *in vacuo*, the residue was extracted into ethyl acetate and treated with TFA/DCM to cleave the Boc group. The TFA/DCM was evaporated *in vacuo* and the product (OCTY-ATMP) was lyophilized from dioxane/water. Ethylenediaminetetraacetic acid (0.25 equivalent EDTA) was coupled with OC2Y-ATMP trifluoroacetate (1 equivalent) in DMF, using BOP as coupling agent in the presence of DIEA. The solvent was removed *in vacuo* and the  
30 residue was purified by HPLC.

5 **EXAMPLE XVII** - Assay of anti-bradykinin activity on guinea pig ileum

Male Hartley guinea pigs that had been deprived of food overnight were sacrificed, and sections of terminal ileum, 25 mm in length, were dissected, attached to tissue holders and immersed in 10 ml tissue baths containing Krebs' solution bubbled with 95%O<sub>2</sub>/5%CO<sub>2</sub>. Tissues were placed under 1 g tension and incubated for 1 h equilibration. Concentration-effect curves were constructed to bradykinin in the absence and presence of new compounds. Bradykinin showed pD<sub>2</sub> = 7.4, and antagonist B9430 showed pA<sub>2</sub> = 7.9.

**EXAMPLE XVIII** - Assay of anti-bradykinin activity on cloned human B2 receptors

Chinese hamster ovary cells containing cloned and expressed human bradykinin B2 receptors were grown in cell cups of the Cytosensor microphysiometer in Ham's F-12 medium supplemented with sodium pyruvate and 10% FBS (Gibco 11765-054). For assay the cells were transferred to Ham's F-12 without bicarbonate or serum (Gibco 21700-075) and placed in the Cytosensor. Concentration-response curves were constructed to bradykinin in the presence or absence of new compounds. Bradykinin showed pD<sub>2</sub> = 11, and antagonist B9430 showed pA<sub>2</sub> = 10.5

**EXAMPLE XIX** - Colorimetric tetrazolium assay for cell survival

Cell growth and survival were measured by a rapid colorimetric assay based on the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Mosmann, *J. Immunol. Methods* 65: 55-63, 1983, with minor modifications). Briefly, 1,000 normal lung fibroblasts or normal epithelial BEAS-2B cells, 1,000 or 5,000 viable non-SCLC cells or 10,000 viable SCLC cells were plated in 100 µL of growth medium in 96-well flat-bottomed microtiter plates. Cells were incubated overnight to allow recovery. Compounds to be tested were added to the cells in triplicate in a range of concentrations and the cells were incubated at 37°C, 5% CO<sub>2</sub>, with 100% humidity. Control cells were treated in the same way without antagonists. All wells had a final volume of 200 µL. Plates were incubated for 4 days, allowing



5 sufficient time for cell replication and compound-induced cell death to occur. On day 5, 25  $\mu$ L of  
a 2 mg/mL solution of MTT (Sigma) dissolved in RPMI-1640 was added to each well. The plate  
was incubated for 4 h at 37°C. The supernate was removed and the blue formazan complex was  
dissolved by adding 100  $\mu$ L of 0.02 N HCl in 75% isopropanol to all wells. Absorbance was  
immediately determined using a scanning multiwell plate reader. B9870 caused 50% cell death at a  
10 concentration of 0.15  $\mu$ M under these conditions.

#### EXAMPLE XX - Measurement of apoptosis in cultured cells

Apoptosis, also known as programmed cell death, is the phenomenon by which a cell dies  
following a series of gene-mediated events, in response to a wide range of intracellular and  
extracellular agents. Apoptosis, a counterpart of mitosis, plays an important role in the  
development and homeostasis of many organisms and tissues. It serves to regulate cell numbers,  
to shape developing organisms and as a defense against potentially harmful agents. Apoptosis is  
not the only mode of cell death. Necrosis is a type of cell death which is nonspecific and  
frequently occurs when cells are exposed to high doses of toxic agents. Such exposure usually  
results in the loss of ionic homeostasis. Unlike apoptosis, necrosis does not seem to be  
genetically influenced.

Apoptotic and necrotic cells have different appearances which allow them to be  
distinguished microscopically. Necrotic cells and their mitochondria swell, the cell membrane  
eventually ruptures, and internal organelles become distended. As a result of the membrane  
25 rupture, inflammation occurs in the surrounding tissue. In contrast, the nuclei of apoptotic cells  
become fragmented into several smaller nuclear bodies, which are quickly recognized by  
phagocytes and engulfed, and no inflammatory response occurs. Therefore, it is useful to  
develop chemotherapeutics which induce apoptosis, rather than necrosis, in order to avoid  
inflammation and the toxic agents which are often released from necrotic tumor cells.

30 We have used differential fluorescent dye uptake and cellular morphology to distinguish  
viable and dead cells with apoptotic and/or necrotic morphologies. We have used Rhodamine 123

10035662-1E801  
20

5 to stain active mitochondria in viable cells, Hoechst 33324 to stain DNA in both viable and dead cells, and Propidium Iodide to stain DNA in dead cells. These cell subpopulations may be distinguished by the different manners in which they take up the fluorescent probes. The dead apoptotic and necrotic subpopulation, which has lost its membrane potential and organelle function, takes up Propidium Iodide and Hoechst 33324. Since the cells in this subpopulation are dead, the mitochondria are not active and thus there is little or no uptake of Rhodamine 123. Under the fluorescence microscope with a DAPI filter, nuclei in these cells appear pinkish in color due to the mixing of both Propidium Iodide and Hoechst 33324 dyes. Necrotic cells have intact nuclei while apoptotic cells have fragmented multi-nucleated bodies. In contrast, the viable apoptotic subpopulation has an intact membrane but inactive mitochondria. As a result, the fragmented multi-nucleated bodies (a hallmark of apoptotic cells) in these cells take up only Hoechst 33324, which gives them a blue appearance under the fluorescence microscope, but are unable to take up Propidium Iodide or Rhodamine 123. The subpopulation of viable cells has both intact cell membranes and active mitochondria. These cells take up both Hoechst 3324 and Rhodamine 123. Microscopically these cells appear to have single normal blue nuclei when examined with a DAPI filter and bright green mitochondria when examined with a FITC filter.

**EXAMPLE XXI – Inhibition of tumor growth *in vivo* in nude mice**

Representative peptide and non-peptide compounds having high *in vitro* cytotoxic activity were tested against implanted tumors *in vivo*. Athymic nude mice were implanted subcutaneously with either single cell suspensions (2 million SCLC cells or 1 million NSCLC cells) or with small fragments (3 x 3 mm) of tumors minced from previously grown nude mouse heterotransplants. On the seventh day after tumor implantation groups of 5 mice bearing implants were injected intraperitoneally daily with the compounds being tested at 1, 5, or 10 mg/kg/day; control animals were injected with an equal volume of isotonic saline. Tumor size was measured with a caliper three times per week. Tumor volume was calculated by the formula:

$$\text{Volume (cc)} = \pi \times (\text{length}) \times (\text{width})^2 / 6$$

- 5 Results of representative *in vivo* tests are given in Figs. 1-8. For comparison, bradykinin antagonist peptide dimers B9870 and B10054 caused marked inhibition of growth of the SCLC line SHP-77 at a dose of 5 mg/kg/day.

#### EXAMPLE XXII - Data

- 10 Examples of peptides and peptide mimics related to the C-terminal part of bradykinin antagonist peptides and their biological activities on cancer cells and bradykinin responses are given in Table 1.

Many compounds not directly related to the structure of bradykinin were synthesized and tested for anti-tumor and anti-bradykinin activity. These are listed in Table 2.

Cyclic peptides related to bradykinin and bradykinin mimics are reported in Table 3, along with their biological activity on cancer cells and anti-bradykinin activity.

Structures of previously described known peptides which have been found to be active against cancers *in vivo* are included in Table 4.

Cytotoxic activity *in vitro* of compounds M570 and M590 against various standard strains of prostate cancer is reported in Table 5.

Standard abbreviations were used for natural amino acids. For non-natural amino acids, derivatizing groups and other chemicals, the abbreviations listed in Table 6 are used.

5 Table 1. ACTIVITIES OF PEPTIDES RELATED TO BRADYKININ STRUCTURE

NUMBER	STRUCTURE	MTT <sup>a</sup>	GPI <sup>b</sup>	HUM AN <sup>c</sup>
BK <sup>d</sup>	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg			
B9430 <sup>d</sup>	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	120	8.2	
B9870-2 <sup>d</sup>	SUIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg)	0.15	8.4	
B8838	DArg-Arg-Pro-Hyp-Gly-CpG-Ser-DCpG-CpG-Arg	--	7.0	
B8840	DArg-Arg-Pro-Hyp-Gly-Phe-Ser-DCpG-CpG-Arg	--	6.8	
B8858	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-CpG-DCpG-DArg	--	5.2	
B8994	DArg-Arg-Pro-MeP-Gly-CpG-Ser-DCpG-CpG-Arg	--	--	
B9074	Dhq-DArg-Arg-Pro-Hyp-Gly-CpG-Ser-DCpG-CpG-Arg	--	6.3	
B9126	Aaa-DArg-Arg-Pro-Hyp-Gly-(D,L)DMF-Ser-DTic-Oic-Arg	--	6.4	
B9126-2	Aaa-DArg-Arg-Pro-Hyp-Gly-(D,L)DMF-Ser-DTic-Oic-Arg	--	7.3	
B9224-2	Aca-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-(D,L)lgl-Oic-Arg	--	8.4	
B9882	α-Sub-Lys(eFlu)-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	--	--	
	<sup>1</sup> DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	--	--	
B9914	Oic-Arg	--	Wk	
B9916	Dlgl-Oic-Arg	--	Wk	
B9490	Dcg-Dlgl-Oic-Arg	>60	Wk	
B9918	Ser-Dlgl-Oic-Arg	--	--	
B9920	Igl-Ser-Dlgl-Oic-Arg	--	--	
B9922	Gly-Igl-Ser-Dlgl-Oic-Arg	--	Wk	
B9924	Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	--	--	
B9926	Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	--	--	
B9950	α-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	8	--	
	Sub-Arg-DNMF-DTrp-Phe-DTrp-Leu			
B9956	α-DDD-(Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg) <sub>2</sub>	--	--	
B9960	DArg-Arg-Nig-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	--	7.7	
B9966	DArg-Arg-NMF-Hyp-Gly-Thi-Ser-Dlgl-Oic-Arg	--	6.9	
B10010	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Nc7G-Arg	--	7.7	
B10014	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nc6G-Arg	--	7.6	
B10054	DDD-(Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg) <sub>2</sub>	0.3	7.1	
B10062	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg-NH <sub>2</sub>	Inact	7.1	
B10082	SUIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg-NH <sub>2</sub> ) <sub>2</sub>	0.7	7.2	
B10084	BAPG-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	>20	8.1	
B10088	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg-Eac-Eac-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Dlgl-Oic-Arg	4	7.1	

B10092	(Gun) <sub>2</sub> -BAPG-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	>20	8.7
B10098	(DArg-Arg-Pro-Hyp) <sub>2</sub> -Dpr-Igl-Ser-DIgl-Oic-Arg	20	5.3
B10100-2	TDIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg) <sub>2</sub>	1	8.0
B10100-1	Moti-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	4	7.8
B10104-2	TDIM-(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg) <sub>2</sub>	4	8.0
B10104-3	Moti-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg	20	8.1
B10160	Leu-DTrp-Phe-DTrp-DNMF-Eac <sub>2</sub> -DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	10	6.3
B10162	Leu-Leu-DTrp-Phe-DTrp-DNMF-Eac <sub>2</sub> -DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	7	6.3
B10198-1	DDD-(Eac-Arg-DIgl-Oic-Arg) <sub>2</sub>	--	5.7
B10198-2	DDD-(Eac-Arg-DIgl-Oic-Arg) <sub>2</sub>	15	--
B10200	DDD-(Eac-Eac-Arg-DIgl-Oic-Arg) <sub>2</sub>	16	5.8
B10238	F5c-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	150	8.1
B10252	EGS-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg) <sub>2</sub>	25	8.3
B10282	Arg-Pro-Pro-Gly-Phe-Thr-DTic-Oic-Arg	--	7.3
B10284	Arg-Pro-Pro-Gly-Phe-Thr-DTic-Oic-NH <sub>2</sub>	--	7.7
B10382	DArg-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg	--	--
B10384	DNiK-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg	--	--
B10386	DDD-(DmK-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg) <sub>2</sub>	--	--
B10388	DNiK-PzO-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg	--	--
B10390	DNiK-PzO-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg	--	--
B10392	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-PFF-Arg	--	--
B10394	F5c-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-PFF-Arg	--	--
B10396	F5c-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-PFF-Arg	--	--
M2	Dcg-D-2-Nal-Arg	--	--
M10	Dcg-2Nal-Arg	--	4.8
M20	Gun-2-Nal-Arg	--	4.6
M42	Gun-Eac-DIgl-Oic-Arg	--	5.0
M68	Dcg-BtA-Arg	--	4.9
M70	Dcg-Igl-Arg	--	4.8
M78	Dcg-Apa-Arg	--	5.6
M84	Dcg-Ile-Arg	--	5.1
M86	Dcg-Ac6c-Arg	--	5.0
M88	Gun-Ica-Arg	--	4.7
M94	Dcg-Aic-Arg	--	Wk
M96	Dcg-(D,L)Ate-Arg	--	4.7
M118-1	Ac-PaF(Mcg)-Arg	--	4.9
M118-3	Ac-PdF-Arg	--	5.4
M124	Dcg-Gly-Cmp-Arg	--	4.7
M128	Dcg-Gly-Oic-Arg	--	Wk
M130	Dcg-F5F-Arg	20	4.8
M132	F5bz-F5F-Arg	60	Wk
M134	Dcg-Trx-Arg	--	4.9

M142	Ac-PaF(Sin)-Arg	--	Wk	
M146-1	Ac-PaF(Mcg)- <i>p</i> -ABz-Arg	--	Inact	
M146-2	Ac-PaF(Dcg)- <i>p</i> -ABz-Arg	--	4.9	
M148	F5c- <i>p</i> -ABz-Arg	--	5.1	
M160	Ste-2-Nal-Arg	--	Wk	
M176	F5c- <i>p</i> ABz-2Nal-Arg	Inact	5.4	--
M196	F5c-Gly- <i>m</i> ABz-2Nal-Arg	Inact	5.1	--
M198	Ac-Pac-Gly- <i>m</i> -Abz-2-Nal-Arg	--	5.1	
M200-1	Mcg-Pac-Gly- <i>m</i> -ABz-2-Nal-Arg	--	Inact	
M200-2	Dcg-Pac-Gly- <i>m</i> -ABz-2-Nal-Arg	--	4.9	
M216	F5c- <i>p</i> -APa-Arg	>180	--	
M226	DDD-(Arg-Digl-Oic-Arg) <sub>2</sub>	35	5.7	Inact
M232-1	Deg-Atpc-Arg	--	4.7	
M232-3	Deg-2-Nal-Atpc-Arg	--	5.1	
M346	Deg- <i>p</i> -Amb-Arg	--	4.6	
M348	F5c- <i>p</i> -Amb-Arg	--	4.7	
M352	F5c- <i>p</i> -Amb-APa-Arg	--	4.7	
M370	F5c-Arg	--	4.8	
M372	F5c-APb-Arg	--	4.6	
M374	Tfmc-Arg	--	4.6	
M380	F5c-Tyr-Arg	--	Inact	
M382	F5c-Tic-Arg	--	4.7	
M388	F5c-Lys((CH <sub>3</sub> ) <sub>3</sub> )-Arg	--	4.9	
M392	F5c-Ana-Arg	--	4.5	
M394	F5c-Bip-Arg	--	4.7	
M398	F5c-Pac-Arg	--	Inact	
M400	DDD-( <i>p</i> ABz-2Nal-Arg) <sub>2</sub>	22	5.1	11.5
M406	Arg-Eac-Digl-Ana-Arg	--	Inact	
M410	F5c-Phe-Arg	--	5.4	
M412	F5c- <i>m</i> -APa-Arg	--	5.8	
M416	F5c-3-Pal-Arg	--	Wk	
M420	F5c-hPhe-Arg	60	7.0	10.9
M424	F5c-Thi-Arg	--	4.6	
M426	F5c-Trp-Arg	--	Inact	
M442	F5c-Oic-Arg	--	--	
M446	F5c-2Nal-Arg	60	4.7	9
M450	F5c-2Nal-Arg-NH <sub>2</sub>	26	4.9	Inact
M484	DDD-(Pac-2Nal-Arg) <sub>2</sub>	25	Inact	Inact
M494	DDD-(Lys-Pac-Gly- <i>m</i> ABz-2Nal-NH <sub>2</sub> ) <sub>2</sub>	33	5.1	Inact
M498	DDD-(Pac-2Nal-Arg-NH <sub>2</sub> ) <sub>2</sub>	24	4.9	Inact
M500	DDD-( <i>p</i> ABz-2Nal-Arg-NH <sub>2</sub> ) <sub>2</sub>	40	0	11.4
M504	DDD-(Pac-2Nal-DArg-NH <sub>2</sub> ) <sub>2</sub>	11	5.4	Wk
M508	DDD-(DArg-2Nal-Arg) <sub>2</sub>	23	Inact	--
M510	DDD-(DArg-2Nal-Arg-NH <sub>2</sub> ) <sub>2</sub>	8	Inact	11
M512	F5c-OC2Y-Arg	70	5.7	11
M516	DDD-(DArg-Arg-Aud-Pac-2Nal-Arg) <sub>2</sub>	1.4	0	Ag
M518	DDD-(DArg-OC2Y-Arg) <sub>2</sub>	15	Wk	10
M520	F5c-OBS-Arg	Inact	6.1	7
M528	F5c-MBC-Arg	Inact	Inact	
M540	Py-a-hPhe-Arg	>100	Wk	
M542	Dca-hPhe-Arg	80	Wk	

M550	F5c-OBT-Arg	80	Inact	Ag
M552	DDD-(p-ABz-hPhe-Arg) <sub>2</sub>	Inact	Inact	
M554	DDD-(DArg-hPhe-Arg) <sub>2</sub>	--	5.1	
M558	Dcg-hPhe-Arg	100	Wk	
M560	DDD-(DArg-hPhe-Arg-NH <sub>2</sub> ) <sub>2</sub>	50	Wk	
M564	DDD-(DArg-OBS-Arg) <sub>2</sub>	--	Wk	
M590	Atmp-Igl-Pac-α-Sbl-Lys-B9430	4.5	7.5	Inact
M598	DDD-(Arg-DIgl-Oic-Arg-OMe) <sub>2</sub>	13	--	10
M600	α-DDD-(Lys-B9430-OMe) <sub>2</sub>	1.2	6.4	Ag
M608	DDD-(Eac-Arg-DIgl-Oic-Arg-OMe) <sub>2</sub>	13	--	Inact
M612	F5c-DArg-hPhe-Arg	Inact	Wk	
M676	DDD-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg) <sub>2</sub>	--	--	
M682	F5c-Lys(F5bz)-Arg	--	4.9	
M686	F5c-NMF-Arg	29	5.6	
M688	F5c-Dpr(Fbz)-Arg	--	Inact	
M690	F5c-Dpr(Paa)-Arg	--	4.8	
M692	DDD-(DArg-Arg-Aud-Pac-hPhe-Arg) <sub>2</sub>	--	Wk	
M696	F5c-DArg-Eac-2Nal-Arg	Inact	5.1	--
M698	F5c-DArg-Arg-Aud-Pac-2Nal-Arg	7.1	Wk	--
M706	Cin-hPhe-Arg	Inact	Wk	
M708	Ppa-hPhe-Arg	Inact	5.8	
M710	DDD-(DArg-Arg-Aud-Pac-2Nal-DArg-NH <sub>2</sub> ) <sub>2</sub>	1.7	Wk	
M714	F5c-PCF-Arg	Inact	Wk	
M718	F5c-PFF-Arg	9	5.1	
M720	F5c-PaF(Ppa)-Arg	Inact	4.8	
M726	D-Arg-Arg-Aud-PaF(F5c)-Arg	Inact	5.6	
M728	DDD-(DArg-Arg-Aud-PaF(F5c)-Arg) <sub>2</sub>	4	5.3	
M730	F5c-DhPhe-Arg	--	4.7	
M732	F5c-PNF-Arg	--	4.9	
M734	DDD-(DArg-Arg-Aud-Pac-PaF(Fbz)-Arg) <sub>2</sub>	1.8	5.3	
M738	F5c-DArg-Eac-hPhe-Arg	Inact	5.3	
M746	DDD-(Pac-hPhe-Arg) <sub>2</sub>	Inact	Inact	
M752-2	Pac-hPhe-Arg	Inact	Wk	
M752-5	Aaa-Ser-Pac-hPhe-Arg	Inact	Wk	
M752-6	Aaa-Pac-hPhe-Arg	Inact	Wk	
M754	Aaa-DPhe-hPhe-Arg	Inact	4.6	
M756	DDD-(DPhe-hPhe-Arg) <sub>2</sub>	18	5.2	
M758	Saa-hPhe-Arg	--	Wk	
M764	Aaa-DTic-hPhe-Arg	--	4.8	
M766	F5c-DArg-Arg-Aud-DTic-hPhe-Arg	--	5.1	
M770	DDD-(DArg-Arg-Aud-DTic-hPhe-Arg) <sub>2</sub>	8	Inact	
M772	Aaa-DIgl-hPhe-Arg	--	4.9	
M774	F5c-DArg-Arg-Aud-DIgl-hPhe-Arg	8	Inact	
M776	DDD-(DIgl-hPhe-Arg) <sub>2</sub>	30	5	
M778-1	Pcc-hPhe-Arg	--	Wk	
M780	Mca-hPhe-Arg	--	Wk	
M782	Cca-hPhe-Arg	--	Wk	
M784	Ac-OC2Y-Arg	Inact	Wk	
M786	DDD-(DArg-Arg-Aud-DIgl-hPhe-Arg) <sub>2</sub>	3.2	Wk	
M788	F5c-DArg-Arg-Aud-DTic-Oic-Arg	9	5	
M790	DDD-(DArg-Arg-Aud-DTic-Oic-Arg) <sub>2</sub>	1.7	Inact	

M792	F5c-DArg-Arg-Eac-Ser-DTic-Oic-Arg	>100	4.9
M794	DDD-(DArg-Arg-Eac-Ser-DTic-Oic-Arg) <sub>2</sub>	21	Inact
M796	F5c-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	31	6.5
M802	F5c-Lys-Ser-DF5F-Oic-Arg	Inact	6.3
M804	DDD-(DArg-Arg-Eac-Ser-DF5F-Oic-Arg) <sub>2</sub>	7.3	7.7
M806	Ava-Igl-Ser-DF5F-Oic-Arg	Inact	5.6
M808	DDD-(Lys-Ser-DF5F-Oic-Arg) <sub>2</sub>	30	6.9
M810	F5c-F5F-Arg	40	4.6
M812	F5c-PFF-Arg-NH <sub>2</sub>	15	Wk
M814	Ppa-PFF-Arg	Inact	Wk
M816	Dpa-PFF-Arg	52	4.6
M818	DDD-(DArg-PFF-Arg-NH <sub>2</sub> ) <sub>2</sub>	60	Wk
M820	DDD-(DArg-PFF-Arg) <sub>2</sub>	43	Inact
M822	DDD-(DArg-F5F-Arg) <sub>2</sub>	25	Mixed
M826	F5c-MFF-Arg	76	Inact
M828	F5c-3,4F2F-Arg	--	Wk
M838	F5c-DArg-Arg-Aud-DIgl-PFF-Arg	7.4	5.1
M842	DDD-(DArg-Arg-Aud-DIgl-PFF-Arg) <sub>2</sub>	1.4	Inact
M844	DArg-Arg-Aud-DIgl-PFF-Arg	12	Wk
M846	DDD-(DArg-Arg-Aud-DF5F-Oic-Arg) <sub>2</sub>	2	7.1
M852	F5c-DArg-Arg-Eac-Ser-DIgl-Oic-Arg	Inact	5.9
M854	DDD-(DArg-Arg-Eac-Ser-DIgl-Oic-Arg) <sub>2</sub>	7.3	5.9
M856	F5c-DArg-Arg-Aud-Ser-DIgl-Oic-Arg	21	5.4
M858	DDD-(DArg-Arg-Aud-Ser-DIgl-Oic-Arg) <sub>2</sub>	4	6.3
M860	F5c-DArg-Arg-Add-Ser-DIgl-Oic-Arg	6	5.4
M862	DDD-(DArg-Arg-Add-Ser-DIgl-Oic-Arg) <sub>2</sub>	1.3	5.6
M864	DDD-(DArg-Arg-Add-Ser-DIgl-PFF-Arg) <sub>2</sub>	1.8	Inact
M868	Ac-DArg-Arg-Aud-DF5F-Oic-Arg	55	6.5
M888	F5c-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	12.5	6.6
M890	DDD-(DArg-Arg-Aud-Ser-DF5F-Oic-Arg) <sub>2</sub>	1.7	5.5
M922	DDD-(DNIK-Arg-Eac-Ser-DF5F-Oic-Arg) <sub>2</sub>	--	--
M926	ζ-SUB-(ApC-F5F-Arg) <sub>2</sub>	Inact	--
M930	α-DDD-(ApC-F5F-Arg) <sub>2</sub>	Inact	--
M932	DDD-(DArg-Arg-Eac-Ser-DIgl-PFF-Arg) <sub>2</sub>	6.0	--
M936	DDD-(DNIK-PzO-Eac-Ser-DF5F-Oic-Arg) <sub>2</sub>	--	--
M944	DDD-(DArg-Arg-Eac-Ser-DF5F-PFF-Arg) <sub>2</sub>	6.7	--
M946	F5c-DArg-Arg-Eac-Ser-DF5F-PFF-Arg	--	--
M950	α-DDD-(K-DArg-Arg-Eac-Ser-DF5F-Oic-Arg) <sub>2</sub>	6.7	--
M952	DDD-(DmK-DArg-Arg-Eac-Ser-DF5F-Oic-Arg) <sub>2</sub>	--	--
M954	Aaa-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	10	--
M956	Aaa-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	14	--
M958	F5bz-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	18	--
M960	Aca-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	21	--
M964	33Dp-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	4-8	--
M968	Dmac-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	15	--
M972	F5pa-DArg-Arg-Aud-Ser-DF5F-Oic-Arg	--	--
M974	DDD-(PzO-F5F-Arg) <sub>2</sub>	--	--
M976	DDD-(DNIK-F5F-Arg) <sub>2</sub>	--	--
M978	DDD-(DPzK-F5F-Arg) <sub>2</sub>	--	--



M980	DDD-(DPzO-F5F-Arg) <sub>2</sub>	--	--
M1024	SUB-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg) <sub>2</sub>	14	7.2
M1026	DTP-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg) <sub>2</sub>	70	6.9
M1028	SBEC-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg) <sub>2</sub>	28	6.7
M1030	EGS-(DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg) <sub>2</sub>	51	7.0
M1034	DDD-(DArg-F5F-DArg-NH <sub>2</sub> ) <sub>2</sub>	--	--
M1036	DDD-(DArg-F5F-DArg) <sub>2</sub>	40	5.4
M1038	e-SUB-(Lys-DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg) <sub>2</sub>	--	6.4
M1042	Aca-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	--	--
M1044	Gun <sub>2</sub> -BArg-DArg-Arg-Eac-Ser-DF5F-Oic-Arg	--	--
M1046	(F5c-DArg-Igl-Arg) <sub>2</sub> -DDA	--	--

5

# Footnotes:

<sup>a</sup> ED<sub>50</sub> for killing of SCLC strain SHP-77 *in vitro*, μM.

<sup>b</sup> pA<sub>2</sub> for bradykinin antagonist activity on isolated guinea pig ileum. The pD<sub>2</sub> of bradykinin is 7.4 on ileum. Higher numbers indicate higher potency.

<sup>c</sup> pA<sub>2</sub> for bradykinin antagonist potency on cloned human B2 receptors, pM. The pD<sub>2</sub> for bradykinin is 11. Higher numbers indicate higher potency.

<sup>d</sup> Data included for comparison<sup>c</sup>

Inact = inactive; Mixed = showing both agonist and antagonist activity; Wk = weak

Table 2. ACTIVITIES OF COMPOUNDS NOT RELATED TO BRADYKININ

NUMBER	STRUCTURE	MTT <sup>a</sup>	GPI <sup>b</sup>	HUMAN <sup>c</sup>
B9948	Arg-DNMF-DTrp-Phe-DTrp-Leu	2.8	Wk	
B10222	DNMF-DTrp-Phe-DTrp-LeuΨ(CH <sub>2</sub> NH)Leu-NH <sub>2</sub>	6.0	5.2	
B10224-1	α-DDD-(Lys-DNMF-DTrp-Phe-DTrp-LeuΨ(CH <sub>2</sub> NH)Leu-NH <sub>2</sub> ) <sub>2</sub>	13	--	
B10224-2	α-DDD-(Lys-DNMF-DTrp-Phe-DTrp-LeuΨ(CH <sub>2</sub> NH)Leu-NH <sub>2</sub> ) <sub>2</sub>	7	--	
B10228	DDD-(DNMF-DTrp-Phe-DTrp-LeuΨ(CH <sub>2</sub> NH)Leu-NH <sub>2</sub> ) <sub>2</sub>	40	Wk	
B10242	Arg-Pro-Lys-Pro-DTrp-Gln-DTrp-Phe-DTrp-LeuΨ(CH <sub>2</sub> NH)Leu-NH <sub>2</sub>	40	5.6	
B10244	DArg-Arg-Pro-Lys-Pro-DTrp-Gln-DTrp-Phe-	12	5.4	

10035662-122801

B 10246	DTrp-LeuΨ(CH <sub>2</sub> NH) <sub>2</sub> Leu-NH <sub>2</sub> DArg-Pro-Lys-Pro-DTrp-Gln-DTrp-Phe-DTrp- LeuΨ(CH <sub>2</sub> NH) <sub>2</sub> Leu-NH <sub>2</sub>	12	6.1	
>278	F5c-Iqa-Atmp	9	5.3	--
M8	Gun-Eac-D2Nal-PgF	--	Inact	
M12	Dcg-Igl-Aqu	20	5.0	--
M18	Dcg-2Nal-Aqu	30	6.6	--
M26	Gun-2Nal-GaP	--	4.9	
M30	Dcg-2Nal-Apa	--	5.4	
M32	Gun-2Nal-Apa	--	4.8	
M36	Dcg-D2Nal-Apa	--	5.0	
M38	Gun-D2Nal-Apa	--	4.9	
M62	Dcg-2Nal-Ama	--	4.8	
M64	Dcg-2Nal-APa-Sud	--	Ag	
M72-1	Dcg-Igl-Apa	--	4.7	
M72-2	Dcg-Igl-APa(anisyl)	--	4.6	
M76	Dcg-2Nal-mABz	--	4.9	
M92-1	Dcg-2Nal-mA <sub>2</sub> Bz	--	5.0	
M92-2	Dcg-2Nal-mA <sub>2</sub> Bz(Gun)	--	4.8	
M92-4	Dcg-2Nal-mA <sub>2</sub> Bz(Dcg)	--	5.0	
M104	Dcg-2Nal-3Pal	--	4.9	
M112	Dcg-D2Nal-mABz	--	5.1	
M120	Dcg-2Nal-pABz	--	4.7	
M122-1	Mcg-APa-mABz	--	5.0	
M122-2	Dcg-APa-mABz	--	4.6	
M136	Sin-F5F-3Pal	--	Inact	
M162	Dcg-2Nal-Asp	--	4.9	
M168-1	2Nap-PaF(Mcg)	--	4.8	
M168-2	2Nap-PaF(Dcg)	--	4.7	
M172	Inp-Dpr(Dcg-2Nal)	--	4.9	
M174	Dcg-Asp-Aqu	--	Inact	
M180	F5c-pABz-2Nal	--	5.1	
M188B	Dcg-2Nal-Asp(Aqu)	--	5.5	
M202	F5c-Gly-mABz-2Nal	--	5.1	
M204	Ac-Pac-Gly-mABz-Nal	--	5.0	
M218	2Nal-Atmp	Inact	4.8	
M222	Dcg-2Nal-Atmp	15	6.8	Inact
M228-2	Dcg(Me)-2Nal-Atmp(Me)	15	7.6	--
M236	Dcg-Igl-Atmp	>50	4.7	--
M240	Dcg-F5f-Atmp	32	5.1	--
M244A	Dcg-2Nal-Atpm	12	5.0	
M244B	Dcg-2Nal-Atpc	Inact	4.9	
M246	Dcg-D2Nal-Atmp	>50	5.7	
M248	F5c-2Nal-Atmp	3.2	6.2	--
M250	Aca-2Nal-Atmp	--	5.2	
M252	Dhq-2Nal-Atmp	Inact	4.8	

M254	TDIM-(2Nal-Atmp) <sub>2</sub>	5	5.1	--
M254-1	TDIM-(2Nal-Atmp) <sub>2</sub>	5	5.1	
M254-2	TDIM-(2Nal-Atmp) <sub>2</sub>	5	5.8	
M258	Dcg-Igl-Atmp	--	5.0	
M262	Dcg-2Nal-Atmp	6	5.3	
M264	Dcg-Trp-Atmp	16	4.7	--
M266	Dcg-Apa-Atmp	42	4.8	--
M268	F5c-2Nal-Tpac	10	5.2	--
M270	Dcg-2Nal-Tpac	--	6.1	
M272	Dpa-2Nal-Atmp	9	5.2	--
M274	Sin-2Nal-Atmp	36	4.7	--
M276	Dca-2Nal-Atmp	4.6	5.3	--
M280	TDIM-(Igl-Atmp) <sub>2</sub>	6	5.2	--
M280-1	Ctim-Igl-Atmp	21	Wk	
M280-2	TDIM-(Igl-Atmp) <sub>2</sub>	6	5.2	
M286	Dtp-(2Nal-Atmp) <sub>2</sub>	24	5.1	--
M288	Boc-2Nal-Atmp	Inact	5.2	--
M288A	Boc-2Nal-Atmp	>85	5.2	
M290-1	Btac-(2Nal-Atmp) <sub>2</sub>	>60	Wk	
M290-2	Btac-(2Nal-Atmp) <sub>3</sub>	20	Wk	
M292	Pac-Igl-Atmp	40	Wk	
M294	DDD-(Pac-Igl-Atmp) <sub>2</sub>	1.8	Inact	10.3
M296	Pya-Bip-Atmp	15	Wk	
M302	Atcp-2Nal-Atmp	3.5	5.2	
M304	TDIM-(2Nal-Dmm) <sub>2</sub>	4.2	5.9	--
M306	Gbz-2Nal-Atmp	>100	--	
M308	Pac-2Nal-Atmp	>75	5.0	
M310	DDD-(Pac-2Nal-Atmp) <sub>2</sub>	1.2	5.1	Inact
M312	Tfmc-2Nal-Atmp	3.2	5.5	--
M314	F5c-2Nal-Aqd	25	4.6	
M316	F5c-Tyr-Atmp	50	--	
M318	F5c-Tyr(Bzl)-Atmp	3.6	5.1	--
M320	F5c-Oic-Atmp	13	--	--
M322	F5c-Tic-Atmp	7.6	--	--
M324	Dmac-2Nal-Atmp	3	5.2	--
M336-1	Dcg-2Nal-Asp-(R,S)Aqu	--	5.1	
M336-2	Dcg-2Nal-Asp-(R,S)Aqu	--	5.4	
M340	Dcg-Pac-Gly-mABz-2Nal	--	4.8	
M342	Dcg-2Nal-Asp-Atmp	--	5.4	
M350	Dcg-2Nal-Glu-Atmp	--	5.0	
M354	Dcg-2Nal-PgF	--	5.2	
M362	Dcg-pAPa-Asp-Atmp	--	4.9	
M364	F5c-pAPa-Asp-Atmp	--	4.7	
M368	Tfmc-pAPa-Asp-Atmp	--	4.7	
M396	F5c-2Nal-Cys(SO <sub>3</sub> H)-Atmp	--	5.0	
M408	Pya-2Nal-Cyh	22	4.6	11.5
M418	F5c-BtA-Atmp	3.8	7.0	10.3
M422	Pya-pABz-2Nal	52	--	Inact
M428	Pya-Gly-mABz-Aqd	>300	--	
M430	DDD-(BtA-Atmp) <sub>2</sub>	18	5.3	11.3
M432	DDD-(2Nal-Asp-Atmp) <sub>2</sub>	70	--	10
M436-1	TDIM-BtA-Atmp	8	4.8	

10035662-122601

M436-2	TDIM-(BtA-Atmp) <sub>2</sub>	4.5	5.8	Ag
M438	F5c-3Pal-Atmp	26	4.8	10
M440	Dcg-BtA-Atmp	30	--	
M448	Dmac-BtA-Atmp	2.7	5.7	Ag
M456	F5c-Cys(Meb)-Atmp	4.7	5.3	Inact
M460	DDD-(3Pal-Nal-Cyh) <sub>2</sub>	15	Wk	
M466	F5c-2Nal-3Ampy	Inact	Wk	
M470	F5c-2Nal-Ampz	11	5.2	Inact
M472	Dmac-2Nal-Ampz	25	5.3	Inact
M474	Pya-2Nal-3Abza	35	Wk	Inact
M476	Tha-BtA-Atmp	15	4.6	Inact
M478	Dmac-2Nal-Thm	30	5.1	Inact
M480-1	HOOC-DDD-Pac-2Nal-Ampz	45	5.1	
M480-2	DDD-(Pac-2Nal-Ampz) <sub>2</sub>	--	5.6	
M492	F5c-mABz-2Nal-Ampz	45	5.1	Inact
M506	Mse-Pac-BtA-Atmp	11	4.9	--
M526	F5c-2Nal-Dmp	10	5.4	Inact
M536	F5c-2Nal-Dmab	4	Wk	Inact
M538	DDD-(Pac-2Nal-Dmp) <sub>2</sub>	>80	Wk	
M568	F5po-2Nal-Atmp	10	5.8	Ag
M570	F5c-OC2Y-Atmp	1.8	5.6	Ag
M572	Dca-2Nal-Acep	2.6	Wk	Wk
M574	Dns-Tyr(Bzl)Atmp	4.5	--	Inact
M582	Dmac-OC2Y-Atmp	3	5.4	9.5
M584-A	DDD-[DArg(Tos)-2Nal-Atmp] <sub>2</sub>	5	Inact	10.3
M584-B	DDD-(DArg-2Nal-Atmp) <sub>2</sub>	5	5.7	11.3
M586-A	Mse-Pac-Igl-Atmp	15	5.3	12
M586-B	Seb-Pac-Igl-Atmp	40	Wk	12.3
M588	α-DDD-(Lys-DArg-2Nal-Atmp) <sub>2</sub>	9.4	Wk	10
M592	F5c-OC2Y-Matp	1.5	4.9	Ag
M594	F5c-MC2Y-Atmp	3.7	5.0	8
M594	F5c-MC2Y-Atmp	3.7	5.0	8
M596-A	DDD-[Arg(Tos)-2Nal-Atmp] <sub>2</sub>	15	5.0	
M596-B	DDD-(Arg-2Nal-Atmp) <sub>2</sub>	8.2	Wk	Inact
M602	Chc-OC2Y-Atmp	12	--	10.8
M604	Pac-2Nal-Ecap	43	4.5	
M606	DDD-(Pac-2Nal-Api) <sub>2</sub>	30	5.0	10
M614	F5c-(N-Dmb)-Tyr(Bzl)-OMe	9.1	Wk	
M616	DDD-(Pac-1Nal-Atmp) <sub>2</sub>	1.4	5.4	--
M618	F5c-DArg-2Nal-Arg-Matp	18	--	--
M620	DDD-(DArg-2Nal-Arg-Matp) <sub>2</sub>	2.0	5.5	--
M622	F5c-OC2Y-Mapp	1.2	5.7	--
M624	Dns-OC2Y-Matp	1.4	5.1	--
M626	Pya-OC2Y-Matp	3.7	4.8	--
M628	Cin-OC2Y-Matp	1.6	5.2	--
M630	Dmac-OC2Y-Matp	1.6	5.0	--
M632	Atcp-OC2Y-Matp	1.4	5.4	--
M636	DDD-(DArg-Arg-Aud-Pac-2Nal-Atmp) <sub>2</sub>	1.7	5.8	--
M638	DDD-(DArg-Igl-Arg-Matp) <sub>2</sub>	0.6	Inact	
M640	DDD-(DArg-BtA-Arg-Matp) <sub>2</sub>	3.0	5.9	
M648	F5c-PaF(Mes)-Atmp	Inact	5.0	
M650	Atcp-OC2Y-Mapp	3.7	--	--

M652	Ppa-OC2Y-Mapp	7.5	5.7	--
M654	Sul-Atmp	Inact	4.5	
M656	Sul-2Nal-Atmp	13	5.4	
M660	DDD-(His-1Nal-Atmp) <sub>2</sub>	30	Wk	--
M662	F5c-tLeu-Atmp	Inact	5.2	--
M664	F5c-OC1Y-Matp	1.2	5.0	
M666	Dns-OC1Y-Matp	1.3	5.0	
M668	SBEC-(DArg-2Nal-Arg-Matp) <sub>2</sub>	3.4	5.2	
M670	DTP-(DArg-Igl-Arg-Matp) <sub>2</sub>	Inact	5.1	
M672	HDD-(DArg-Igl-Arg-Matp) <sub>2</sub>	--	--	
M674	DDD-(DArg-F5F-Arg-Matp) <sub>2</sub>	3.5	Wk	
M678	(Dns-DArg-Igl-Arg) <sub>2</sub> -DDA	1.1	5.3	
M724	F5c-DArg-Aud-OC2Y-Gly-Atmp	12	5.4	
M744	DDD-(DArg-2Nal-Arg-Dmab) <sub>2</sub>	3.4	5.3	
M798	F5c-OC2Y-Dmab	37	--	
M800	DDD-(DArg-OC2Y-Dmab) <sub>2</sub>	27	5.3	
M832	F5c-PFF-Dmab	47	4.6	
M834	DDD-(DArg-PFF-Arg-Dpea) <sub>2</sub>	1.6	5.3	
M848	DDD-(DArg-F5F-Arg-Dmab) <sub>2</sub>	--	--	
M880	DDD-(DArg-F5F-Arg-Dpea) <sub>2</sub>	--	--	
M886-1	DDD-DArg-PFF-Arg-NH <sub>2</sub>	3.2	Wk	
	L-DArg-PFF-Arg-Dpma			
M886-2	DDD-(DArg-PFF-Arg-Dpma) <sub>2</sub>	--	Inact	
M892	DDD-(DArg-PFF-Arg-PFF-NH <sub>2</sub> ) <sub>2</sub>	8.5	Wk	
M900	DDD-(DArg-F5F-Arg-PaF-NH <sub>2</sub> ) <sub>2</sub>	6.3	--	
M916	F5c-DArg-PFF-Arg-PFF-NH <sub>2</sub>	5.7	4.9	
M1032	DDD-(DArg-Igl-Mapp) <sub>2</sub>	15	5.4	
M1040	EDTA-(OC2Y-Atmp) <sub>4</sub>	0.73	--	

## Footnotes:

<sup>a</sup>ED<sub>50</sub> for killing of SCLC strain SHP-77 *in vitro*,  $\mu$ M.

<sup>b</sup>pA<sub>2</sub> for bradykinin antagonist activity on isolated guinea pig ileum. The pD<sub>2</sub> of bradykinin is 7.4 on ileum. Higher numbers indicate higher potency.

<sup>c</sup>pA<sub>2</sub> for bradykinin antagonist potency on cloned human B2 receptors, pM. The pD<sub>2</sub> for bradykinin is 11. Higher numbers indicate higher potency.

Ag = agonist; Inact = inactive; Wk = weak

5 Table 3. ACTIVITIES OF CYCLIC PEPTIDES

NUMBER	STRUCTURE	MTT <sup>a</sup>	GPI <sup>b</sup>
B9458-2	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-CpGΨ(CH <sub>2</sub> N)Arg   CO CH <sub>2</sub> / \	--	6.1
B9462	Darg-Arg-Pro-Hyp-Gly-Thi-Ser-Digl-CpGΨ(CH <sub>2</sub> N)Arg   CO CH <sub>2</sub> / \	7.3	6.0
B10302	CH <sub>2</sub> CO <sub>2</sub> H c[DArg-Arg-Pro-Hyp-Gly-Igl-Ser-Digl-Oic-Arg]	Inact	5.2
B10304	Aca-c[DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Nig-Oic-Arg]	Inact	6.4
B10306	c[Arg-DNMF-DTrp-Phe-DTrp-Leu]	Inact	Wk
B10312	α-DDD-(c[Lys-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DF5F-Oic-Arg]) <sub>2</sub>	3.8	--
M680	c[DArg-Arg-Eac-Ser-DF5F-Nc7G-Arg]	--	--
M824	c[Ava-Igl-Ser-DF5F-Oic-Arg]	Inact	5.2
M850	c[DArg-Arg-Aud-Digl-PFF-Arg]	1.4	5.1
M868-2	c[DArg-Arg-Aud-DF5F-Oic-Arg]	9.2	6.1
M870	c[DArg-Arg-Add-DF5F-Oic-Arg]	5.5	5.3
M872	c[DArg-Arg-Eac-Ser-DF5F-Oic-Arg]	2.2	Inact
M874	c[DArg-Arg-Add-Ser-DF5F-Oic-Arg]	11	5.0
M876	c[DArg-Arg-Aud-Ser-DF5F-Oic-Arg]	22.5	5.4
M878	c[DArg-Arg Add-Digl-PFF-Arg]	7	Wk
M882	c[DArg-Arg-Add-Ser-Digl-PFF-Arg]	4.5	Inact
M896	c[DArg-Arg-Eac-Digl-PFF-Arg]	65	Wk
M902	c[DArg-Arg-Ava-Ser-Digl-PFF-Arg]	30	5.5
M906	c[DArg-Arg-Eac-DF5F-Oic-Arg]	45	Wk
M908	c[DArg-Arg-Ava-Ser-DF5F-Oic-Arg]	40	4.9
M910	c[Bala-DArg-Arg-Eac-Ser-DF5F-Oic-Arg]	42	5.2
M924	c[Suc-DArg-Arg-Eac-Ser-Digl-PaF]-Arg	37	Wk
M934	c[DNIk-Arg-Eac-Ser-DF5F-Oic-Arg]	--	--
M940	c[DNIk-PzO-Eac-Ser-DF5F-Oic-Arg]	--	--
M986	c[Add-DArg-F <sub>2</sub> F-Arg]	--	--

Footnotes:

10 <sup>a</sup>ED<sub>50</sub> for killing of SCLC strain SHP-77 *in vitro*, μM.

<sup>b</sup>pA<sub>2</sub> for bradykinin antagonist activity on isolated guinea pig ileum. The pD<sub>2</sub> of bradykinin is 7.4 on ileum. Higher numbers indicate higher potency.

Inact = inactive; Wk = weak

10035662.122801

**Table 4. PREVIOUSLY DESCRIBED KNOWN PEPTIDES THAT NEWLY SHOW *IN VIVO* ANTI-CANCER ACTIVITY**

NUMBER	STRUCTURE
B9430	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg
B9330	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg
B10044	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-Oic-Arg
B10050	Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-ChG
B10206	DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DF5F-Nc7G-Arg
B10288	DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg

These compounds showed anti-tumor activity *in vivo* when tested by the procedure of Example XXI.

**TABLE 5. CYTOTOXICITY *IN VITRO* AGAINST STRAINS OF PROSTATE CANCER**

COMPOUND NUMBER	PROSTATE CANCER CELL LINE					SCLC
	DU14	TSU	LNCa	PC-3	PPC1	SHP-77
B9870	0.08	6.5	3.7	3.2	4.3	0.15
M570	1.2	2.8	3.0	1.6	3.0	1.8
M590	0.01	7.0	7.0	6.3	12	4.5

Numbers are ED<sub>50</sub> (μM) for cytotoxic activity. Activity against SCLC strain SHP-77 is included for comparison.

**TABLE 6. ABBREVIATIONS USED FOR COMPOUNDS**

B9430 = DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg  
 B9870 = SUIM-(DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)<sub>2</sub>  
 Aaa = 1-Adamantaneacetyl  
 AAA = amino acid analysis  
 ABz = Aminobenzoic acid  
 ABza = Aminobenzyl alcohol  
 Ac = Acetyl  
 Ac3c = 1-Amino-1-cyclopropanecarboxylic acid  
 Ac5c = 1-Amino-1-cyclopentanecarboxylic acid ("cyclo-Leu")  
 Ac6c = 1-Aminocyclohexanecarboxylic acid  
 Aca = 1-Adamantanecarboxyl  
 Acep = 4-Amino-1-carbethoxymethyl-2,2,6,6-tetramethylpiperidine  
 ADA = 1,3-Adamantanediacyl

Add = 12-Aminododecanoic acid  
 Aib =  $\alpha$ -Aminoisobutyric acid  
 Aic = 2-Aminoindane-2-carboxylic acid  
 ALG =  $\alpha$ -Allylglycine (2-amino-4-pentenoic acid)  
 Ama = Aminomethylanthranilic acid  
 Amb = Aminomethylbenzoic acid  
 Ampy = 3-Aminomethylpyridine  
 Ampz = 1-Amino-4-methylpiperazine  
 Ana = Anthranilic acid  
 APa = *p*-Aminophenylacetic acid  
 APb = *p*-Aminophenylbutyric acid  
 ApC = S-3-Aminopropylcysteine  
 Api = 4-Aminopiperidine  
 Apm = 4-Amino-1,2,2,6,6-pentamethylpiperidine  
 Aptp = 4-Amino-1-phenylmethyl-2,2,6,6-tetramethylpiperidine  
 Aqd = 4-Aminoquinaldine  
 Aqu = 3-Aminoquinuclidine  
 Arg(NO<sub>2</sub>) = Arginine(Nitro)  
 Atc = 2-Aminotetralin-2-carboxylic acid  
 Atcp = 4-Amino-3,5,6-trichloropicolinic acid  
 Atmp = 4-Amino-2,2,6,6-tetramethylpiperidine  
 AtmpO = 4-Amino-2,2,6,6-tetramethylpiperidinyloxy  
 Atpc = 4-Amino-2,2,6,6-tetramethyl-4-piperidinecarboxylic acid  
 Atpm = 4-Amino-4-methoxycarbonyl-2,2,6,6-Tetramethylpiperidine  
     (4-Amino-2,2,6,6-tetramethyl-4-piperidinecarboxylic acid methyl  
     ester)  
 Aud = 11-Aminoundecanoic acid  
 Ava = 5-Aminovaleric acid  
 Azt = Azetidine-2-carboxylic acid  
 BAla =  $\beta$ -Alanine  
 BApG = N,N-bis(3-aminopropyl)-glycine  
 BAPTA = 1,2-bis(2-Aminophenoxy)ethane-N,N,N',N'-tetraacetyl  
 Bip = Biphenylalanine  
 Boc = *tert*-Butoxycarbonyl; [(1,1-dimethylethoxy)carbonyl]  
 BOP = Benzotriazoyloxytris(dimethylamino)phosphonium  
     hexafluorophosphate  
 BPHD = N,N'-bis(2,2,6,6-tetramethyl-4-piperidiny)-1,6-hexanediamine  
 BSH = 1,6-Bissuccinimidohexane  
 BtA = 3-Benzothienylalanine  
 BTAC = Benzene-1,3,5-*tris*-carboxamido-6-caproyl  
 BTC = 1,3,5-Benzenetricarboxyl



Bz = Benzoyl  
 Bzl = Benzyl  
 CAcH = *cis*-2-Amino-1-cyclohexanecarboxylic acid  
 Cca: 2-Chlorocinnamic acid  
 CDF = *p*-Chloro-D-phenylalanine  
 ChA =  $\alpha$ -Cyclohexylalanine  
 Chc =  $\alpha$ -Cyano-4-hydroxycinnamoyl  
 ChG =  $\alpha$ -Cyclohexylglycine  
 CHO = Chinese hamster ovary  
 CHTC = 1,3,5-Cyclohexanetricarboxyl  
 CHyp = *cis*-4-Hydroxy-proline  
 Cin = Cinnamoyl  
 CMeb = S-(4-Methylbenzyl) cysteine  
 CmF = (Z) *p*-Chloro-2,3-methanophenylalanine  
 Cmp = 4-Carboxymethylpiperazine  
 CpA =  $\alpha$ -Cyclopropylalanine  
 CpG =  $\alpha$ -Cyclopentylglycine  
 CpGY(CH<sub>2</sub>N)Arg = CpG pseudo(CH<sub>2</sub>NH) Arg  
 CPTA = *trans*-1,2-Diaminocyclohexane- N,N,N',N'-tetraacetyl  
 CTAC = Cyclohexane-1,3,5-*tris*-carbamido- $\epsilon$ -caproyl  
 Ctim = 13-Carboxytridecanimidyl  
 Cyh = Cyclohexylamine  
 Dabz = Diaminobenzoic acid  
 DArg(NO<sub>2</sub>) = Nitro-Arginine  
 Dca = Dicyclohexylacetyl  
 Deg = N,N'-Dicyclohexylguanidyl  
 DCM = Dichloromethane  
 DDA = 1,10-Decanediamine  
 DDD = Dodecanedioyl  
 DDS = 2-Dodecen-1-ylsuccinyl  
 DEA = N,N'-Diethylethylenediamine  
 DhP = 3,4-Dehydroproline  
 Dhq = 2,3-Dehydroquinuclidine-3-carboxyl  
 DIC = Decahydroisoquinoline-3-carboxylic acid  
 DIEA = Diisopropylethylamine  
 Dmab = 4-Dimethylaminobenzylamine  
 Dmac = 4-Dimethylaminocinnamoyl  
 Dmb = 4-(Dimethylamino)benzyl  
 DmF = 2,4-Dimethylphenylalanine  
 DMF = Dimethyl formamide  
 DmK =  $\epsilon$ -Dimethyllysine

Dmm = 2,6-Dimethylmorpholine  
 Dmp = 3-Dimethylaminopropylamine  
 DmtP = 5,5-Dimethyl-4-thiaproline  
 Dns = Dansyl (5-dimethylamino-1-naphthalenesulfonyl)  
 22Dp = 2,2-Diphenylpropionyl  
 33Dp = 3,3-Diphenylpropionyl  
 Dpa = Diphenylacetyl  
 Dpea = Diphenylethylamine  
 Dpma = Diphenylmethylamine  
 Dpr = 2,3-Diaminopropionic acid  
 DTP = Dithiobis-propionyl  
 DTPA = Diethylenetriaminepentaacetyl  
 Eac =  $\epsilon$ -Aminocaproic acid  
 Ecap = N-Ethoxycarbonyl-4-amino-piperidine (Ethyl 4-amino-1-piperidinecarboxylate)  
 EDA = 4,4'-Ethylenedianiline  
 EDP = 4,4'-Ethylenedipiperidine  
 EDTA = Ethylenediaminetetraacetyl  
 EDTP = Ethylenediaminetetrapropionic acid  
 EGS = Ethylene glycol-*bis*-succinyl  
 EGTA = Ethylene glycol-*bis*-( $\beta$ -aminoethyl ether)- N,N,N',N'-tetraacetyl  
 EOPC = 1,1'-Ethylenebis(5-oxo-3-pyrrolidinecarboxyl)  
 ETTA = 2,2',2'',2'''-[Ethanediylidenetetakis(thio)tetrakisacetyl]  
 F2F = Difluorophenylalanine  
 F5bz = Pentafluorobenzoyl  
 F5c = 2,3,4,5,6-Pentafluorocinnamoyl  
 F5F = Pentafluorophenylalanine  
 F5pa = 2,3,4,5,6-Pentafluorophenylacetyl  
 F5po = 2,3,4,5,6-Pentafluorophenoxyacetyl  
 Fbz = *para*-Fluorobenzoyl  
 Flu = Fluorescein thiourea  
 Gaa = Guanidinoacetyl  
 GaP = 2-Guanidyl-3-(4-aminophenyl)propionic acid  
 Gbz = 4-Guanidinobenzoyl  
 Glt = Glutaryl  
 Gun = Guanidyl  
 HATU = O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate  
 HbQ = 4-Hydroxybutylglutamine  
 HDA = 1,6-Hexanediamine  
 HDD = Hexadecanediol

HF = Hydrogen fluoride  
 HFG = Hexafluoroglutaroyl  
 HiG = Hexahydro-2-indanylglycine  
 HOAt = 1-Hydroxy-7-azabenzotriazole  
 hPhe = Homo-phenylalanine  
 HPLC = high performance liquid chromatography  
 Hxa = Hexanoic acid  
 Hyp = *trans*-4-Hydroxyproline  
 Ica = Indoline-2-carboxylic acid  
 Igl =  $\alpha$ -2-Indanylglycine  
 Ing =  $\alpha$ -1-Indanylglycine  
 Inp = Isonipecotic acid  
 Iq2a = 6,7-Dimethoxy-3,4-dihydro-1-isoquinolineacetic Acid  
 Iq4a = 6,7-Dimethoxy-1,2,3,4-tetrahydro-1-isoquinolineacetic acid  
 Lau = Lauroyl  
 Leu(r)Leu = Leu-pseudo(CH<sub>2</sub>NH)Leu  
 Leu $\Psi$ (CH<sub>2</sub>NH)Leu = Leu-pseudo(CH<sub>2</sub>NH)Leu  
 LDMS = laser desorption mass spectrometry  
 mA<sub>2</sub>Bz = 3,5-Diaminobenzoic acid  
 MaG =  $\alpha$ -Methylglycine (2-amino-3-methyl-4-pentenoic acid)  
 Mapp = 4-(Methylamino)-1,2,2,6,6-pentamethylpiperidine  
 Matp = 4-(Methylamino)-2,2,6,6-tetramethylpiperidine  
 MatpO = 4-(N-methylamino)-2,2,6,6-tetramethylpiperidinylloxy  
 MBC = S-(4-methylbenzyl)cysteine  
 MBHA = Methylbenzhydrylamine  
 MC2Y = N-Methyl-O-2,6-dichlorobenzyl-tyrosine  
 Mca = 2-Methylcinnamic acid  
 Mcg = Monocyclohexylguanidyl  
 Meb = Methylbenzyl  
 MeP = 2,4-Methanoproline  
 Mes = Methanesulfonyl  
 MFE = (E)-2,3-Methanophenylalanine  
 MFF = *meta*-Fluorophenylalanine  
 Mosi = Methoxy-suberimido  
 Moti = 14-Methoxytetradecanediimidoyl  
 Mse = Methoxysebacyl  
 MTT = (3-(4,5)-Dimethyltriazol-2-yl)-2,5-diphenyl tetrazolium bromide  
 Nal =  $\beta$ -Naphthylalanine  
 Nap = Naphthoyl  
 Nba = Norbornane-2-acetyl  
 Nbc = Norbornenedicarboxyl

Nbi = Norbornedicarboximide  
 Nbn = 2-Aminonorbornane-2-carboxylic acid  
 Nc5G = N-Cyclopentylglycine  
 Nc6G = N-Cyclohexylglycine  
 Nc7G = N-Cycloheptylglycine  
 Nc8G = N-Cyclooctylglycine  
 Nig = N-2-Indanylglycine  
 NiK =  $\epsilon$ -Nicotinoyllysine  
 NMF = N-Methylphenylalanine  
 NSCLC = non-small cell carcinoma  
 OBS = O-Benzylserine  
 OBT = O-Benzylthreonine  
 OBY = O-Benzyltyrosine  
 OC2Y = O-2,6-Dichlorobenzyltyrosine  
 OC1Y = O-2,6-Dichlorobenzyl-3,5-diiodotyrosine  
 Oct = Octanoyl  
 Oic = Octahydroindole-2-carboxylic acid  
 OMe = O-Methyl  
 OMY = O-Methyltyrosine  
 OSY = Tyrosine O-sulfate ester  
 Paa = Phenylacetyl  
 Pac = 4-Aminocinnamic acid  
 PaF = *p*-Aminophenylalanine  
 Pal =  $\beta$ -Pyridylalanine  
 Pba = Phenylbutyryl  
 Pcc = *trans*-2-Phenyl-1-cyclopropanecarboxylic acid  
 PCF = *p*-Chlorophenylalanine  
 Pcpa =  $\alpha$ -Phenylcyclopentaneacetyl  
 PdF = *p*-Dicyclohexylguanidylphenylalanine  
 PFF = *p*-Fluorophenylalanine  
 PFS = Perfluorosuberoyl  
 PgF = *p*-Guanidinophenylalanine  
 PheOL = Phenylalaninol  
 PhG = Phenylglycine  
 Pip = Pipecolic acid ("homo-Pro")  
 PipA =  $\beta$ -3-Piperidylalanine  
 PNF = *p*-Nitrophenylalanine  
 Ppa = Phenylpropionyl  
 Pya = *trans*-3-(3-Pyridyl)acryloyl  
 PyAOP = 7-Azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate

PzK =  $\epsilon$ -Pyrazinoyllysine  
 PzO = 4-Pyrazinoylornithine 4-(2-pyrazin carboxyl) orn  
 Saa = *trans*-Styrylacetic acid  
 SBEC = Sulfo-*bis*-ethoxycarbonyl  
 Sbl = Sebacoyl  
 SCLC = small cell lung carcinoma  
 Seb = Sebacyl  
 Sin = Sinapinyl (3,5-dimethoxy-4-hydroxycinnamoyl-)  
 Ste = Stearoyl  
 Sua = Sulfanilamide (4-Aminobenzenesulfonamide)  
 SUB = Suberyl  
 Suc = Succinyl  
 Sud = Sulfadiazine  
 SUIM = Suberimidyl  
 Sul = Sulindac  
 Tba = *t*-Butyl-acetyl  
 TDIM = Tetradecanediiimidyl  
 TEA = Triethylamine  
 TFA = Trifluoroacetic acid  
 Tfmc = *trans*-4-(Trifluoromethyl)cinnamoyl  
 Tha = 3-(2-Thienyl)acryloyl  
 Thi =  $\beta$ -2-Thienylalanine  
 Thm = Thiomorpholine  
 Thz = Thiazolidine-4-carboxylic acid (4-thiaproline)  
 Tic = 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid  
 TLC = thin layer chromatography  
 TLeu = *tert*-Leucine  
 TMF = 2,4,6-Trimethylphenylalanine  
 Tos = *p*-Toluenesulfonyl  
 Tpac = 2,2,5,5-Tetramethyl-3-(aminoethyl)-pyrroline-3-carboxamide  
 TREN = *tris*(2-Aminoethyl)amine  
 Trx = Tranexamic acid (*trans*-4-((Aminomethyl)cyclohexanecarboxylic acid)